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Letter to the Editor

Exclusion of Primary Congenital Glaucoma (Buphthalmos) From Two Candidate Regions of Chromosome Arm 6p and Chromosome 11

To the Editor:

Primary congenital glaucoma (gene symbol: GLC3) is characterized by an improper development of the aqueous outflow system. The reduced outflow of fluid results in an increased intraocular pressure leading to buphthalmos, optic nerve damage, and eventual visual impairment. GLC3 is a heterogeneous condition with an estimated incidence of 1:2,500 in Middle Eastern and 1:10,000 in Western countries. In many families, GLC3 is an autosomal recessive trait with presentation of an earlier age-of-onset, high intraocular pressure, enlarged cloudy cornea, buphthalmos, and a more aggressive course.

The pathogenesis of GLC3 remains elusive despite extensive histologic efforts to identify a single anatomic defect. Recent advances in positional mapping and cloning of human disorders provided an opportunity to identify chromosome locations of the GLC3 phenotype. Our laboratory is currently involved in the mapping of this condition by using a combination of candidate chromosome regions associated with the GLC3 phenotype and by a general positional mapping strategy.

GLC3 has been repeatedly reported in the literature to be associated with various types of chromosomal abnormalities [Schinzel, 1984]. Among these, 2 major chromosome regions have merit as a candidate for site of the GLC3 locus.

First, GLC3 has been associated with ring chromosome 6 [Levin et al., 1986]. Zurcher et al. [1990] reviewed the literature and demonstrated an apparent association of congenital eye anomalies with distal del(6p) and r(6). The anomalies described in their report include GLC3, hyperopia, anterior chamber defect, megalocornea, iris abnormalities, and optic atrophy. They concluded that deletion of the distal portion of 6p is associated with ocular dysmorphogenesis. Subsequently, Hafez et al. [1990] reported an association between GLC3 and the HLA system at 6p21. Furthermore, they suggested that a GLC3 gene is linked to the HLA system and showed a strong linkage disequilibrium with the HLA-B8 haplotype. This study focused attention on the 6p21 region and away from the telomeric portion of 6p. Recently molecular deletion of DNA markers from the 6p25 region is reported in 2 different cases of GLC3 carrying translocations [Nishimura et al., 1994, 1995]. This group also indicated that at least one locus for GLC3 may reside in this region of 6p. Their molecular evidence strongly suggests that 6p25 is more likely to be the site of GLC3 than 6p21. In order to resolve these discrepancies with respect to the site of GLC3 on chromosome 6, we embarked on a genetic linkage analysis of series of markers that are located on 6p.

Second, a family with pericentric inversion of chromosome 11 (p15::q12) was described in which all cytogenetically affected members in 2 generations had GLC3 [Braughton et al., 1983]. This report is unique showing familial segregation of a chromosome abnormality and GLC3. However, with the exception of one case [Ohnishi et al., 1990], no eye malformation has ever been reported in patients with a pericentric inversion of chromosome 11 [Simola et al., 1977; Autio-Hermainen and de la Chapelle, 1980]. It is also interesting that the GLC3 locus has initially appeared on chromosome 11 in the gene map section of the OMIM (On Line Mendelian Inheritance in Man) and subsequently was repeated in different morbidity maps that were published from the OMIM database [McKusick, 1993; McKusick and Amberger, 1993].

In view of the above-mentioned reports, we decided to genotype our families with PCRable markers from 6p and 11. We have so far identified a total of 80 GLC3 families from Turkey and Canada with a total of 261 potential informative offspring. Details of diagnostic criteria and comprehensive clinical findings of our families were published elsewhere [Turacli et al., 1992; Barsoum-Homsy and Chevrette, 1986]. All patients in our families have only primary GLC3 without any other associated abnormalities. In order to reduce the chance of heterogeneity and to ensure the recessive mode of inheritance in our initial screening panel, we preselected a group of 19 Turkish families that have a high degree of consanguinity and a large number of sibs otherwise unavailable in the Canadian families. This family panel consists of a total of 99 offspring, of whom 45 are affected with primary GLC3 (Table I). Further 2 kindreds had to be excluded from this panel as one is likely to have a pseudodominance mode of inheritance (family 17) and the other is only sampled partially (family 23).

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TABLE I. Pedigree Structure of Families Segregating for Primary GLC3

Pedigree no.	1	2	4	6	7	10	11	13	14	15	16	17	18	19	20	21	22	23	24	
Consanguineous	Y	N	Y	N	Y	Y	Y	Y	N	Y	Y	Y	N	N	Y	N	N	Y	Y	Sum
Affected sibs	2	3	4	2	2	2	4	2	2	3	3	2	2	2	2	2	2	2	2	45
Normal sibs	6	1	9	1	3	3	5	0	1	1	2	4	7	1	1	2	1	6	0	54
Total sibs	8	4	13	3	5	5	9	2	3	4	5	6	9	3	3	4	3	8	2	99
Sibs sampled	7	4	11	3	5	5	8	2	3	4	5	2	7	3	3	4	3	0	2	81

For the purpose of linkage evaluation, we selected 12 markers from 6p that cover the critical regions as summarized above and another 8 markers from chromosome 11. The positional information of the markers was obtained from previously published maps [Gyapay et al., 1994; Buetow et al., 1994]. We used polymerase chain reaction (PCR), denaturing polyacrylamide gel electrophoresis, and silver staining to genotype our families. Lod scores were calculated using the MLINK function of the LINKAGE package (FASTLINK version). Calculations were carried out under the assumption of autosomal recessive inheritance and complete penetrance. The results of 2-point linkage analysis and the exclusion area for each of the markers studied are summarized in Table II.

So far, we have excluded regions 6p21 and 6p25 as the likely site for the GLC3 locus (Table II). The deletion detected in the 6p25 region of the reported GLC3 individual is within an estimated distance of about 5 cM that is flanked by markers D6S344 and D6S477. We genotyped our families for D6S477 and excluded an 18 cM region on both sides of this marker as a candidate site for the GLC3 locus (Table III). Of the 17 kindreds genotyped for this marker, 2 were uninformative and only one kindred with 3 offspring (pedigree no. 6, Table III) showed no recombination with D6S477. Of a total of 72 informative offspring for this marker, only these 3 did not show any recombination with D6S477 and therefore this observation can be attributed to chance alone. Thus, it is not unlikely that even this family is unlinked to this region of 6p. Therefore, our study clearly shows that the apparent association between GLC3 and the deletion of the markers at 6p25 is coincidental, or this is due to the existence of genetic heterogeneity between different geographic populations. Similarly, haplotype analysis as well as the obtained negative lod scores with markers from 6p21 and the entire chromosome 11 exclude these regions as a likely site of the GLC3 locus. Hence, we conclude that information contained in the OMIM and those previously published do not represent an accurate location of the GLC3 locus on 6p or 11. We are currently searching the entire genome, aiming to identify the location of a GLC3 locus.

TABLE II. Lod Scores Between Primary GLC3 and DNA Markers on 6p and 11

	Recombination fractions						
Marker	.001	0.05	0.10	0.20	0.30	0.40	Z # 2 2
D6S477	2 41.4	2 10.10	2 5.38	2 1.74	2 0.47	2 0.07	0.18
D6S1006	2 9.8	2 1.63	2 0.51	0.18	0.23	0.09	0.04
D6S105	2 34.6	2 8.19	2 4.23	2 1.21	2 0.23	2 0.01	0.16
D6S273	2 19.3	2 4.45	2 2.23	2 0.58	2 0.08	0.01	0.10
D6S306	2 11.0	2 2.67	2 1.39	2 0.39	2 0.06	0.01	0.07
TUBB	2 14.2	2 2.66	2 1.03	0.01	0.17	0.07	0.06
D6S1014	2 29.4	2 6.52	2 3.21	2 0.79	2 0.07	0.03	0.13
D6S497	2 24.1	2 4.53	2 1.77	0.07	0.36	0.16	0.09
D6S291	2 28.5	2 5.71	2 2.51	2 0.36	0.09	0.03	0.11
D6S1019	2 12.8	2 1.44	0.08	0.86	0.68	0.24	0.04
D6S1017	2 16.0	2 2.92	2 1.08	0.14	0.29	0.11	0.07
D6S1018	2 5.3	2 0.62	2 0.09	0.10	0.05	0.01	0.01
D11S1981	2 23.4	2 5.30	2 2.74	2 0.78	2 0.16	2 0.01	0.12
D11S1397	2 19.4	2 4.60	2 2.49	2 0.83	2 0.24	2 0.04	0.12
D11S1392	2 44.4	2 12.70	2 7.68	2 3.23	2 1.22	2 0.28	0.25
D11S1393	2 26.9	2 7.10	2 4.03	2 1.50	2 0.49	2 0.10	0.17
D11S1975	2 9.9	2 1.80	2 0.76	2 0.07	0.05	0.02	0.04
D11S1396	2 36.9	2 10.20	2 6.06	2 2.46	2 0.90	2 0.20	0.22
D11S1986	2 26.1	2 6.40	2 3.49	2 1.25	2 0.43	2 0.10	0.15
D11S1982	2 17.1	2 4.00	2 2.13	2 0.68	2 0.18	2 0.03	0.10

TABLE III. Individual Family Lod Scores Between Primary GLC3 and D6S477 on 6p25*

	Recombination fractions (cM)									
Pedigree no.	0.001	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
1	−4.47	−1.17	−0.67	−0.41	−0.26	−0.16	−0.09	−0.05	−0.02	−0.01
2	−2.40	−0.72	−0.44	−0.29	−0.19	−0.13	−0.08	−0.04	−0.02	−0.00
4	−4.80	−1.44	−0.89	−0.59	−0.39	−0.25	−0.15	−0.08	−0.04	−0.01
6	0.72	0.62	0.51	0.41	0.31	0.22	0.14	0.08	0.04	0.01
7	−4.80	−1.44	−0.89	−0.59	−0.39	−0.25	−0.15	−0.08	−0.04	−0.01
10	−1.72	−0.12	0.07	0.14	0.16	0.14	0.11	0.07	0.04	0.01
11	−6.90	−1.91	−1.12	−0.70	−0.45	−0.28	−0.16	−0.09	−0.04	−0.01
14	−1.97	−0.35	−0.13	−0.03	0.01	0.03	0.03	0.02	0.01	0.00
15	−1.80	−0.19	0.02	0.10	0.12	0.12	0.10	0.06	0.03	0.01
18	−2.14	−0.50	−0.26	−0.15	−0.08	−0.05	−0.02	−0.01	−0.00	−0.00
19	−2.10	−0.46	−0.23	−0.12	−0.06	−0.03	−0.01	−0.00	−0.00	0.00
20	−2.40	−0.72	−0.44	−0.29	−0.19	−0.13	−0.08	−0.04	−0.02	−0.00
21	−2.15	−0.50	−0.25	−0.13	−0.07	−0.03	−0.01	−0.00	−0.00	0.00
22	−2.10	−0.46	−0.23	−0.12	−0.06	−0.03	−0.01	−0.00	−0.00	0.00
24	−2.40	−0.72	−0.44	−0.29	−0.19	−0.13	−0.08	−0.04	−0.02	−0.00
Sum	−41.42	−10.10	−5.38	−3.07	−1.74	−0.94	−0.47	−0.21	−0.08	−0.02

* Total exclusion area = 18 cM; lod score value = −2.19.

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